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Precision and Accuracy in the Determination of Organics in Water by Fused Silica Capillary Column Gas Chromotography/Mass Spectrometry and Packed Column Gas Chromatography/Mass Spectrometry

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16. ABSTRACT

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Precision and Accuracy in the Determination of Organics in Water by Fused Silica Capillary Column Gas Chromatography/Mass Spectrometry and Packed Column Gas Chromatography/Mass Spectrometry

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Two general methods for the identification and measurement of organic compunds in water are compared. One method employs packed column chromatography and the other fused silica capillary column chromatography. The two gas chromatography/mass spectrometry (GC/MS) methods use different pH conditions for the liquid–liquid extractions with methylene chloride. Single laboratory total method accuracy and precision data are presented for over 80 analytes in water at the low parts per billion level. With the packed column method, 54% of the analytes have a mean recovery of 70% or more. With the capillary column method, 74% of the analytes have a mean recovery of 70% or greater. Both methods give mean relative standard deviations for concentration measurements of about 20%.

Concentration measurements with gas chromatography/mass spectrometry (GC/MS) are strongly identified with the selected ion monitoring (SIM) method of data acquisition. The scope, precision, and sensitivity of SIM have been thoroughly reviewed recently (*1*). In many applications, for example, the broad spectrum analysis of environmental samples (*2*) or the measurement of a long list of target compounds in a complex sample matrix, the preferred data acquistion method is the repetitive measurement of spectra as separated components emerge from the chromatograph. This technique requires a computer data acquisition system for the practical storage and retrieval of all the data. Storage and retrieval of complete mass spectra are required to allow their examination, which permits reliable identifications of the components separated from often complex mixtures. A number of investigators have recognized (*3–5*) the potential efficiency (cost-effectiveness) of making concentration measurements with the same mass spectral data stored in the data system files.

The purpose of this report is to present data which define the scope and limitations of this type of simultaneous identification and measurement process. The scope and limitations are presented in terms of the total method precision and accuracy for a classical liquid–liquid partition, extract concentration, and repetitive scanning GC/MS analysis. The test compounds selected for study were from a group that are of environmental interest but are not sufficiently volatile for the inert gas purge and trap method (*5, 6*). The liquid–liquid extraction from water utilized methylene chloride, which was considered the general purpose solvent with the most favorable properties and fewest disadvantages (*7*). The GC/MS results were obtained with both conventional packed and wall-coated fused silica capillary columns. Two variations of the general method are designated method 625(8) and method 625.1 for convenient reference.

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EXPERIMENTAL SECTION

Materials. The compounds used in this study were the highest purity materials available from commercial sources and were used without purification. Typically 100.0 mg of each compound was dissolved in a separate 100-mL portion of acetone to form a series of stock solutions. For benzidine and 3,3′-dichlorobenzidine the solvent was methanol; for most polycyclic aromatic hydrocarbons with a molecular weight greater than 250 the solvent was benzene. Mixtures of analytes were prepared by combining aliquots of the individual stock solutions and diluting the mixture with acetone. Generally aliquots in the range of 0.3–3.0 mL were diluted to 10 mL or 100 mL to give analyte concentrations in the range of 10–30 ng/μL. Some analytes which were mixtures of many compounds and several compounds which displayed poor chromatographic behavior were used at higher concentrations. Benzidine and 3,3′-dichlorobenzidine in methanol were added to the mixture immediately before use to preclude losses by reactions of these analytes with acetone. The standard solutions of mixtures of analytes were used for standard additions to the water matrix and to calibrate the GC/MS systems. All solvents were distilled in an all-glass apparatus within 24 h of their use. Reagent water was prepared by passing distilled water through a column containing about 1 lb of granular activated carbon.

Instrumentation for Method 625. Analyses were carried out with a Varian Model 1400 gas chromatograph interfaced to a Finnigan Model 3200 mass spectrometer with an all-glass transfer line and an all-glass jet-type enrichment device. All compounds except those of the phenolic group were chromatographed with a 1.8 m × 2 mm i.d. glass tube packed with 3% SP-2250 on 100/120 mesh Supelcoport. Compounds of the phenolic group were chromatographed with a 1.8 m × 2 mm i.d. glass tube packed with 1% SP-1240DA on 100/120 mesh Supelcoport. The columns were placed in the chromatograph oven so that the injection end was in contact with the septum to facilitate on-column injection which was used throughout the study. The injector temperature was maintained at 260 °C and the transfer line at 240 °C. The carrier gas was helium at a flow of 30 mL/min. The spectrometer sweep was controlled by an interface to a Digital Equipment Corp. Model PDP-8/E computer operating under revision E0 of the System 150 operating system. Data acquired were stored in disk files under the disk operating system and displayed via a cathode ray rube (CRT), a printer, or an incremental plotter. All data were collected at a nominal 70 eV in the electron impact ionization mode. The temperature of the ion source was unknown but probably exceeded 70 °C.

Instrumentation for Method 625.1. Analyses were carried out with a Finnigan Model 9610 gas chromatograph interfaced to a Finnigan Model 4000 mass spectrometer. All compounds were chromatographed with a 28 m × 0.25 mm i.d. fused silica capillary column coated with SE 54. The column was extended through the interface oven and into the spectrometer ion source slightly without intercepting the electron beam. The chroma-

Table I. Accuracy and Precision for Measurements of Substituted Benzenes with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (m/z)	C(M), μg/L	R	mean recovery, %	S, %	C(M), μg/L	R	mean recovery, %	S, %
1,2-dichlorobenzene	146	3.8	5	105	122	10	7	61	10
1,3-dichlorobenzene	146	3.8	5	50	31	10	7	58	6.1
1,4-dichlorobenzene	146	13.3	7	37	4.6	10	7	68	11
1,2,4-trichlorobenzene	180	3.8	5	86	23	10	7	76	11
1,2,3-trichlorobenzene	180	4	8	58	9.9	not measured			
1,3,5-trichlorobenzene	180	4	8	40	5.8	not measured			
1,2,3,4-tetrachlorobenzene	214	4	8	59	12	not measured			
1,2,3,5-tetrachlorobenzene	214	4	8	50	9.1	not measured			
pentachlorobenzene	248	4	8	55	9.8	not measured			
hexachlorobenzene	284	3.8	5	73	18	10	7	98	24
nitrobenzene	123	3.8	5	91	40	10	7	6	6.5
2,4-dinitrotoluene	165	13.3	7	35	3.1	10	7	79	11
2,6-dinitrotoluene	165	3.8	5	70	21	10	7	116	19

tograph was equipped with a Grob-type splitless injector which was used throughout the study. The injector and interface oven temperatures were maintained at 260 °C. The carrier gas was helium at a linear velocity of 28 cm/s. The spectrometer sweep was controlled by an interface to a Data General Model Nova 3 computer operating under revision 3.1C of the Finnigan-Incos operating system. Data acquired were stored in disk files under the disk operating system and displayed via a CRT or a printer-plotter. All data were collected at a nominal 70 eV in the electron impact ionization mode. The temperature of the ion source was 220 °C.

Procedure for Method 625. Eight 1-L aliquots of reagent water were placed in a series of 2-L separatory funnels. To each of seven aliquots was added 0.2 mL of the mixed standard solution of analytes in acetone. The eighth reagent water aliquot was carried through all subsequent operations as a reagent blank. Alternatively, for some analytes, 2.0 mL of the mixed standard solution of analytes in acetone was added to 10 L of reagent water in a 10-L separatory funnel. After being mixed, 1-L aliquots of the solution were placed in a series of seven 2-L separatory funnels. A separate portion of reagent water was used for the reagent blank. The detailed procedure for extraction and analysis of the water samples has been published (8). Briefly, the analytes were twice partitioned between pH adjusted water and methylene chloride by mixing the two liquid phases in the separatory funnel. The first partition was at pH 11, the second at pH 2, and the methylene chloride extracts were analyzed separately. Both extracts were dried by passing them through a column of purified anhydrous sodium sulfate, concentrated to a low volume with a Kuderna–Danish apparatus, and analyzed by on-column injection into the packed chromatographic columns described under Instrumentation for Method 625. Before analysis, a known quantity of the internal standard anthracene-d_{10} was added to each extract to give a concentration approximately the same as the expected concentrations of the analytes. An aliquot of the extract obtained at pH 11 was injected into the SP-2250 column at 50 °C. This temperature was held for 3 min, allowing the solvent to elute, and then programmed at 8 °C/min to 265 °C. Mass spectrometer data acquisition began with the initiation of temperature programming. The spectrometer was scanned repetitively from 45 to 460 amu at 8 ms/amu, and with a settling time of 2.3 ms/amu, the scan time was 4.3 s/spectrum. The 265 °C temperature was held for approximately 25 min or until the last analyte eluted. An aliquot of the extract obtained at pH 2, which contained the phenols, was injected into the SP-1240DA column at 60 °C. This temperature was held for 3 min, programmed at 6 °C/min to 190 °C, and then held until the last analyte eluted. Again data acquisition began with the initiation of temperature programming.

Procedure for Method 625.1. Mixtures of analytes in reagent water were prepared substantially the same as described under Procedure for Method 625. The extraction procedure was also the same with the single major exception that the first set of methylene chloride–water partitions was at pH 7, the second at pH 2, and the methylene chloride extracts were combined before concentration and analyzed together. Three internal standards were added to the dried concentrated extract before analysis. The internal standards were naphthalene-d_8, anthracene-d_{10}, and chrysene-d_{12}, and they were added to give concentrations approximately the same as the expected concentrations of the analytes. An aliquot of the extract was injected into the fused silica capillary column at 40 °C. This temperature was held for 4 min, and then programmed at 10 °C/min to 275 °C. Mass spectrometer data acquisition began with the initiation of temperature programming. The spectrometer was scanned repetitively from 45 to 460 amu at 0.95 s/spectrum and with a 0.05 s pause at 45 amu. The 275 °C temperature was held for 30 min or until the last analyte eluted.

Calibration and Quantitation. Methods 625 and 625.1 employed very similar single-point calibration procedures. To the mixed standard solutions in acetone, which were used to prepare the water solutions, were added quantities of the internal standards so that the concentrations of analytes and standards were the same or very similar. These calibration standards were analyzed by using the GC/MS conditions described in each procedure. Response factors were computed from the quantitation ion peak areas and the quantities of analytes and internal standards injected. The quantitation ions used are shown in Tables I–VII. The response factors were used, along with the measured extract component peak areas and the known quantities of internal standards added to the extracts, to compute the concentrations of analytes in the water solutions. The response factors were always measured with 4 h of the extract analyses, and the concentrations in the calibration standards were always within a factor of 2 of the concentrations in the extracts. With this procedure the calibration error was minimized. The only difference in the calibration procedures for the two methods was that Method 625 used a single internal standard for all analytes, and Method 625.1 used three internal standards. The analytes in Method 625.1 were divided into three groups by chromatographic retention time, and one internal standard was used for each group. In all cases it was established that the ratio of analyte area to internal standard area was linear with respect to the amount of analyte injected at constant internal standard amount over the short concentration range of concern. With Method 625 quantitation ion peak areas, response factors, and water concentrations were determined by using a System 150 program called Superquan. Details of this program are available from the authors as a separate report. With Method 625.1 quantitation ion peak areas, response factors, and extract concentrations were determined by using the standard Finnigan-Incos program Quan.

Quality Control. A reagent water blank was extracted with each batch of samples that was processed. Generally, no samples were analyzed unless this reagent water was free of all background signals from the measured compounds. In one batch of samples background signals at the quantitation ions for dibutyl phthalate, benzidine, and 3,3′-dichlorobenzidine were detected in the blank, and corrections were made in the measured concentrations of these compounds. The 70-eV mass spectrum of bis(perfluorophenyl)phenylphosphine (DFTPP) was measured at least once each working day by using the gas chromatographic inlet system.

Table II. Accuracy and Precision for Measurements of Substituted Phenols with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (m/z)	C(M), μg/L	R	mean recovery, %	S, %	C(M), μg/L	R	mean recovery, %	S, %
phenol	94	8.3	7	25	7.4	20	7	42	2.3
2-fluorophenol	112	8.3	7	36	11	20	7	55	11
2-chlorophenol	128	8.3	7	51	17	20	7	72	11
2-nitrophenol	139	8.3	7	46	16	20	7	73	10
4-nitrophenol	65[a]	16.3	7	32	3.8	20	7	29	12
2,4-dichlorophenol	162	8.3	7	60	13	20	7	74	12
2,4-dimethylphenol	122	8.3	7	27	7.8	20	7	70	6.2
2,4-dinitrophenol	184	41.6	7	57	18	20	7	67	15
4-chloro-3-methylphenol	142	8.3	7	64	11	20	7	76	14
2,3,6-trichlorophenol	196	8.3	7	66	9.5	not measured			
2,4,6-trichlorophenol	196	not measured				20	7	78	16
3,4,5-trichlorophenol	196	8.3	7	76	4.9	not measured			
2,4,6-trimethylphenol	121	not measured				20	7	66	3.8
2-methyl-4,6-dinitrophenol	198	41.6	7	56	22	20	7	121	23
pentachlorophenol	266	16.6	7	75	8.4	30	7	95	24

[a] The quantitation ion for Method 625.1 was 139.

Table III. Accuracy and Precision for Measurements of Polycyclic Aromatic Hydrocarbons with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (m/z)	C(M), μg/L	R	mean recovery, %	S, %	C(M), μg/L	R	mean recovery, %	S, %
naphthalene	128	3.8	5	106	35	10	7	73	8
acenaphthylene	152	3.8	5	84	50	10	7	76	13
acenaphthene	154	3.8	5	76	20	10	7	91	21
fluorene	166	3.8	5	73	21	10	7	93	19
anthracene	178	3.8	5	75	16	10	7	94	5.9
phenanthrene	178	13.3	8	42	4.7	10	7	91	12
fluoranthene	202	3.8	5	84	15	10	7	99	21
pyrene	202	3.8	5	81	13	10	6	104	25
benz[a]anthracene	228	not measured				10	7	91	24
chrysene	228	3.8	5	89	13	10	7	102	15
benzo[a]pyrene	252	3.8	5	71	13	10	7	66	19
benzo[b]fluoranthene	252	13.3	8	35	7.3	10	7	114	29
benzo[k]fluoranthene	252	3.8	5	82	15	10	7	87	24
benzo[ghi]perylene	276	3.8	5	79	13	20	7	78	32
indeno[1,2,3-cd]pyrene	276	3.8	5	73	13	20	7	82	31
dibenzo[a,h]anthracene	278	3.8	5	79	12	10	6	121	41

The measured spectrum was compared with published criteria (9), and if the criteria were not met, the ion source and other mass spectrometer operating variables were adjusted until an acceptable spectrum was obtained. Chromatographic performance was evaluated with test mixtures of analytes on a daily basis, and no measurements were made unless satisfactory performance was attained.

RESULTS AND DISCUSSION

Tables I–VII show the test analytes used with Methods 625 and 625.1. The methods are defined in the Experimental Section, and the analytes are grouped in the tables according to their structures or other common features. Each table gives for each method and analyte the following information: (a) the quantitation ion; (b) the concentration made, C(M), in water; (c) the number of complete method replicates, R; (d) the mean recovery for the R replicates in terms of the percentage of the C(M); and, (e) the estimated standard deviation (S) of the percentage recovery.

Figure 1 shows the distribution of mean recoveries among Method 625 analytes. Of the 82 analytes tested with Method 625, 44 (54%) had mean recoveries of 70% or greater. Five analytes have zero recovery which is attributed to chemical reactions in water at pH 11. These analytes are all found in Table IV and are chlorinated hydrocarbon pesticides. With Method 625.1, 57 analytes (74%) had mean recoveries of 70% or greater, and the distribution of recoveries is shown in Figure 2. The five analytes which gave zero recovery with Method 625 were all recovered with Method 625.1. A few analytes that were measured at several concentrations were included in the above totals at each level. For several reasons not all analytes were measured with both methods, but 66 different analytes were tested with both methods.

Figure 3 shows the distribution of estimated standard deviations of the mean recoveries among Method 625 analytes. Of the 77 measurable method 625 analytes, 70 (91%) had an estimated standard deviation of 25% or less. Two dichlorobenzenes had particularly variable recoveries (Table I) and the third a low mean recovery. This is partly attributed to their volatility, which leads to losses during extract concentration, but mainly to imprecise peak area measurements caused by poor resolution from the solvent on the packed chromatography column. With Method 625.1, 66 (86%) of the analytes had an estimated standard deviation of the mean recovery of 25% or less (Figure 4). The dichlorobenzenes are well resolved from the solvent on the fused silica capillary column and demonstrated acceptable method precision. Some of the compounds with the most variable recoveries were the higher molecular weight polycyclic aromatic hydrocarbons (Table III), which may not be transferred efficiently and consistently to the column in the splitless injector system.

It is important to recognize that Method 625 was designed for use with two separate packed chromatography columns.

Table IV. Accuracy and Precision for Measurements of Chlorinated Hydrocarbon Pesticides with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (m/z)	C(M), µg/L	R	mean recovery, %	S, %	C(M), µg/L	R	mean recovery, %	S, %
hexachlorocyclohexane (α isomer)	109[a]	5	6	0		10	7	90	18
hexachlorocyclohexane (β isomer)	109[a]	6	10	91	23	10	7	95	7.3
hexachlorocyclohexane (γ isomer)	109[a]	7	6	0		10	7	87	12
hexachlorocyclohexane (δ isomer)	109[a]	6	10	72	27	10	7	92	4.9
1,1-bis(4-chlorophenyl)-2,2-dichloroethane (DDD)	235	10	6	97	6.8	not measured			
1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (DDT)	235	10	6	82	9.7	10	7	85	8.6
1,1-bis(4-chlorophenyl)-2,2-dichloroethene (DDE)	246	10	10	87	18	10	6	104	22
aldrin	66[b]	10	6	86	3.9	10	7	90	5.6
endrin	81	10	10	0		10	7	66	48
dieldrin	79	10	6	96	5.2	10	7	108	20
heptachlor	100[c]	10	6	84	7.5	10	7	110	14
heptachlor epoxide	81[d]	10	6	93	5.9	10	7	119	22
chlordane (γ isomer)	373	not measured				20	7	93	13
endosulfan I	75[e]	6	10	0		10	6	87	51
endosulfan II	195	6	10	0		10	7	107	80
endosulfan sulfate	272	7	10	80	29	10	7	71	11
toxaphene	[f]	not measured				300	6	72	9.1

[a] The quantitation ion for Method 625.1 was 219. [b] The quantitation ion for Method 625.1 was 263. [c] The quantitation ion for Method 625.1 was 272. [d] The quantitation ion for Method 625.1 was 353. [e] The quantitation ion for Method 625.1 was 195. [f] The sum of ion abundance at masses 159, 161, 195, and 197 was used for this measurement (*10*).

Table V. Accuracy and Precision for Measurements of Phthalic Acid Esters, *N*-Nitroso Compounds, and Isophorone with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (m/z)	C(M), µg/L	R	mean recovery, %	S, %	C(M), µg/L	R	mean recovery, %	S, %
dimethyl phthalate	163	3.8	5	40	7.3	10	7	80	10
diethyl phthalate	149	3.8	5	73	15	10	7	118	13
di-*n*-butyl phthalate	149	3.8	5	89	16	10	7	70	14
n-butylbenzyl phthalate	149	3.8	5	71	17	10	7	88	10
di-*n*-octyl phthalate	149	3.8	5	81	15	10	7	70	30
di-2-ethylhexyl phthalate	149	3.8	5	89	8.3	10	7	85	6.4
N-nitrosodimethylamine	74	not measured				100	7	42	14
N-nitrosodi-*n*-propylamine	130	not measured				20	7	67	6.0
N-nitrosodiphenylamine	169[a]	3.8	5	68	14	10	7	89	13
isophorone	82	3.8	5	87	25	10	7	64	14

[a] Measured as diphenylamine because compound decomposes in the injection port.

Table VI. Accuracy and Precision for Measurements of Miscellaneous Halogenated Compounds with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (m/z)	C(M), µg/L	R	mean recovery, %	S, %	C(M), µg/L	R	mean recovery, %	S, %
hexachloroethane	117	3.8	5	76	23	10	7	55	6.2
bis(2-chloroethyl) ether	93	13.3	7	36	4.8	10	7	66	10
bis(2-chloroisopropyl) ether	45	not measured				10	7	67	23
bis(2-chloroethoxy)methane	93	13.3	7	39	5.3	10	7	67	15
hexachlorobutadiene	225	3.8	5	74	18	10	7	64	15
hexachlorocyclopentadiene	237	not measured				10	7	38	7.1
4-chlorophenyl phenyl ether	204	13.3	8	37	7.1	10	7	96	21
4-bromophenyl phenyl ether	248	3.8	5	71	16	10	7	89	21
1-fluoronaphthalene	146	4.0	8	53	5.6	not measured			
1-fluoronaphthalene	146	4.8	5	88	25	not measured			
1-fluoronaphthalene	146	13.3	7	41	4.3	10	7	76	7.9
2-chloronaphthalene	162	3.8	5	78	23	10	7	75	9.1

Table VII. Accuracy and Precision for Measurements of Substituted Biphenyls with Methods 625 and 625.1

		625				625.1			
compound	quantitation ion (m/z)	C(M), µg/L	R	mean recovery, %	S, %	C(M), µg/L	R	mean recovery, %	S, %
4,4′-dibromobiphenyl	312	10	16	91	16	not measured			
4,4′-dibromobiphenyl	312	13.3	8	39	4.4	not measured			
4,4′-dibromobiphenyl	312	36	8	37	4.5	not measured			
4,4′-dibromooctafluorobiphenyl	296	4	8	47	4.2	not measured			
4,4′-dibromooctafluorobiphenyl	296	10	16	88	14	10	7	112	48
Aroclor 1221	188	91	8	61	10	not measured			
Aroclor 1248	294	not measured				50	7	88	5.8
Aroclor 1254	326	91	8	70	10	not measured			
4,4′-diaminobiphenyl(benzidine)	184	not measured				50	7	128	94
4,4′-diamino-3,3′-dichlorobiphenyl	252	33	8	43	14	50	7	154	67

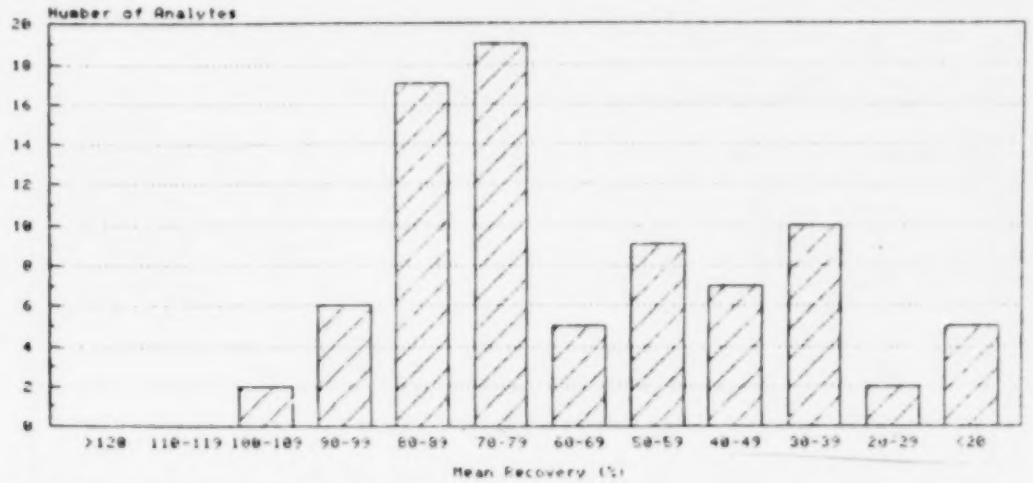

Figure 1. Distribution of mean recoveries with Method 625.

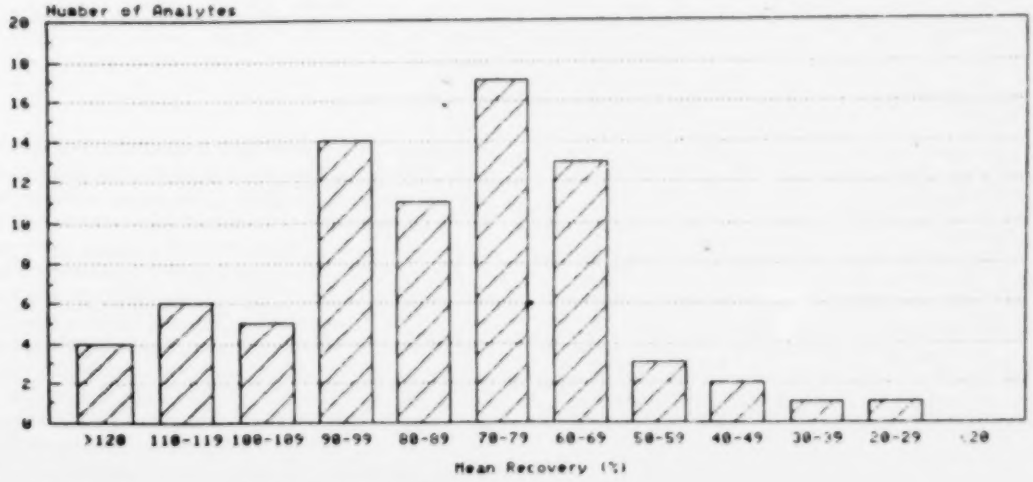

Figure 2. Distribution of mean recoveries with Method 625.1.

One column was selected for optimum separation of a group of phenols (Table II) and the other was selected for optimum separation of a variety of halogenated organics and aromatic compounds (Tables I, III–VII). Therefore, it was necessary to provide a chemical separation of these two groups before chromatography, and liquid–liquid extractions at pH 11 and pH 2 were selected to implement this broad class separation. With the development of capillary columns and particularly fused silica capillary columns capable of superb separations of the compounds in Tables I–VII, the broad class chemical separation became unnecessary. It was desirable to eliminate the pH 11 extraction because it was no longer necessary, and many organic compounds are sensitive to strongly basic conditions (*11*). Therefore, a pH 7 extraction followed by a pH 2 extraction was selected for Method 625.1. The pH 7 extraction minimizes the risk of acid and base catalyzed reactions of analytes. The pH 2 extraction ensures extraction of acidic phenols that may not be extracted efficiently at pH 7. An optional third extraction at pH 12 may be used for very basic compounds that are not extracted efficiently at pH 7.

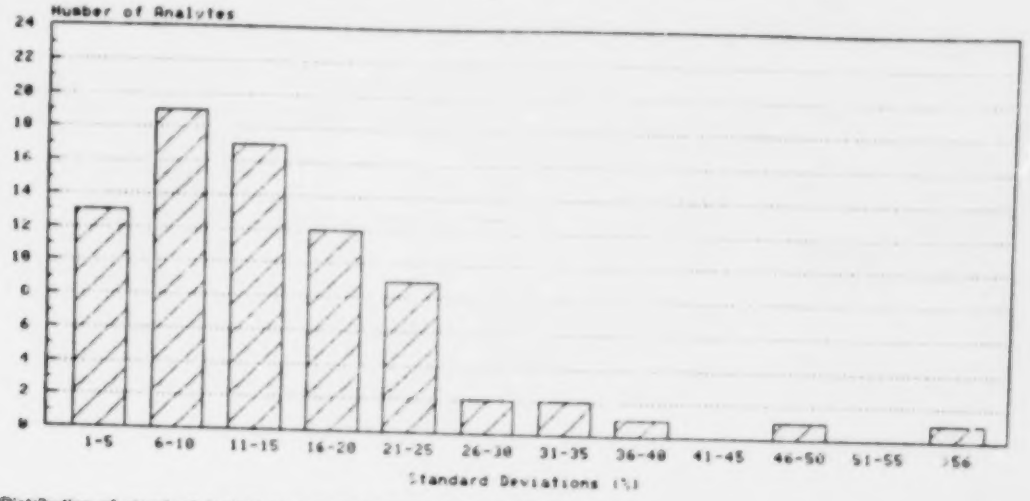

Figure 3. Distribution of standard deviations of mean recoveries with Method 625.

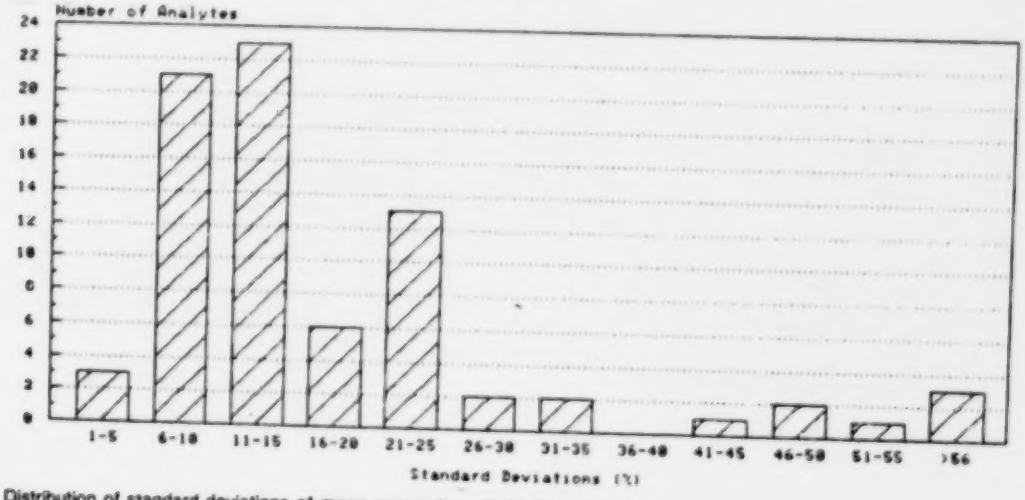

Figure 4. Distribution of standard deviations of mean recoverins with Method 625.1.

This option was not included in Method 625.1 but has been recommended (7). The combined extracts are then chromatographed with the fused silica capillary column.

The fundamental differences in the two methods led to several differences in the way the methods were implemented in this study. The test analytes were studied in batches, and more batches were used with Method 625 because of lower chromatographic resolution with the packed columns. Nearly all analytes were measured in two large batches with Method 625.1 Other significant additional differences were the use of on-column injection and a GC/MS interface enrichment device with Method 625, and the use of Grob splitless injection and no interface device with Method 625.1.

Method Bias. Methods 625 and 625.1 were designed to reveal the extraction and extract processing efficiencies and to allow an evaluation of method bias. With both methods the internal standard is added to the concentrated methylene chloride extract immediately before chromatography. It is assumed there is no bias during injection, chromatography, and mass spectral analysis because any losses of analytes should be compensated with the internal standard calibration technique. Therefore, the measured concentrations may be compared with the amounts added to the water samples to determine the mean percentage recovery or method bias. These values are in Tables I–VII as previously discussed. A major source of low recovery (and variability) is loss of relatively volatile analytes during extract concentration. This is most apparent in the recoveries of the dichlorobenzenes and trichlorobenzenes in Table I, the more volatile phenols in Table II, and isolated relatively volatile compounds in the other tables. Qualitatively there appears to be some improvement in the recoveries of these more volatile compounds with Method 625.1. This may be partly the result of more accurate peak area measurements which are made possible by far better resolution of the sample components from the solvent peak. It was not possible to measure *N*-nitrosodimethylamine (Table V) with Method 625 becuase its signal was obscured by the solvent peak; however, with Method 625.1 resolution was adequate for peak measurement, but losses during extract concentration are still apparent.

Five chlorinated hydrocarbon pesticides (Table IV) gave zero recovery with Method 625, but excellent recoveries with Method 625.1. This is attributed to the base sensitivity of the compounds since each chromatographs well on the packed column. The α and γ isomers of hexachlorocyclohexane each have two chlorines in a trans axial configurstion with hydrogen which is a favorable arrangement for base-catalyzed dehydrochlorination. The δ isomer has similar relationships, but apparently reacts slower with base. Clearly Method 625.1 is the preferred method for the analysis of this group, especially for the γ isomer, which is the active pesticide component in mixtures of these isomers. Similar base sensitivity was ob-

served with the two endosulfan isomers, which are sulfurous acid esters with the sulfur and two oxygens part of a seven-membered ring. Endosulfan sulfate, which has the same general structure but sulfur in the higher oxidation state, was recovered in good yield from the basic solution of Method 625. Perhaps sulfur in the lower oxidation state is more susceptible to attack by hydroxide ion. Endrin, a stereoisomer of dieldrin, was not recovered from the basic solution of Method 625, but dieldrin was recovered in excellent yield.

The compound 4,4′-diaminobiphenyl (benzidine, Table VII) was not measured with Method 625 because its standard solutions in acetone were found to be unstable. Benzidine slowly reacts with acetone to form a monoimine that is readily recognized by its mass spectrum, which contains an intense molecular ion at mass 224 and an M − 15 ion at mass 209. In time, a second compound appeared which was identified as the bis(imine) by its similarly characteristic mass spectrum. Solutions of benzidine in methanol are stable, and these were used in the preparation of water samples for Method 625.1. Nevertheless, the recovery of benzidine and its 3,3′-dichloro derivative were quite erratic as shown in Table VII. It has been reported (*12*) that benzidine is sorbed on sodium sulfate during the drying of organic extracts and is subject to thermal decomposition during Kuderna–Danish (KD) extract concentration. This was confirmed by processing three solutions containing 100 μg of benzidine in methylene chloride through the KD step only. Recoveries were 19, 36, and 69%, and when the drying plus KD step was tested, recoveries dropped to 7, 13, and 26%. It is concluded that benzidine and some of its derivatives are not viable analytes with these methods.

Qualitatively, Table II shows generally higher mean recoveries of phenols with Method 625.1. In addition to the improved resolution of the more volatile phenols from the solvent, a factor discussed previously, the use of a combined extract with Method 625.1 probably contributes to somewhat better recoveries. Some phenols are partitioned into methylene chloride at pH 11, and this reduces their recovery in the pH 2 extract with Method 625. In an independent study, approximately 12% of the 2,4-dimethylphenol and smaller amounts of 4-chloro-3-methylphenol were found in the pH 11 extract. These diversions are often neglected in routine applications of Method 625 and may account for some small part of the lower recoveries.

The compound *N*-nitrosodiphenylamine (Table V) decomposes in a hot gas chromatograph injection port and is measured as diphenylamine. Therefore, this compound cannot be unambiguously measured with the two methods unless they are modified to separate diphenylamine prior to gas chromatography. A similar problem exists with another potential analyte, the benzidine precursor 1,2-diphenylhydrazine, which decomposes to azobenzene in the injection port. Hexachlorocyclopentadiene (Table VI), an important intermediate in the synthesis of many chlorinated bicyclohydrocarbon pesticides, was found susceptible to decomposition at low concentrations in solvents unprotected from light. No data were obtained with Method 625, but when solutions were protected from light, the compound was measurable with Method 625.1. The low recovery of hexachlorocyclopentadiene may be partly accounted for by slow degradation during storage or processing.

In general, Methods 625 and 625.1 are least biased with the stable, higher molecular weight substituted benzenes, phenols, polycyclic aromatic hydrocarbons, chlorinated pesticides, phthalic acid esters, and other halogenated compounds. Two low recoveries of aromatic hydrocarbons reported in Table III with method 625 are assumed due to systematic errors because there are no obvious reasons why these two compounds should behave differently from the others of the group.

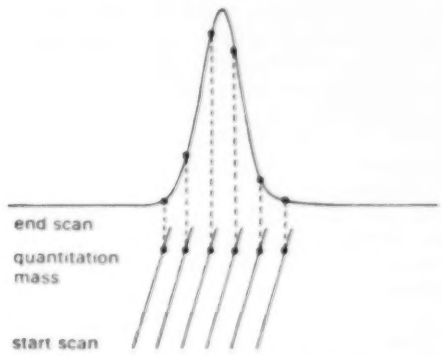

Figure 5. Schematic representation of peak area measurements with data acquisition by repetitive measurement of spectra.

Given this generality, the methods may be extended to numerous classes of compounds with similar properties. Overall Method 625.1 is qualitatively superior to Method 625 and provides generally improved recoveries, especially for phenols, base-sensitive compounds, and the more volatile compounds that are not well resolved from the solvent on a packed chromatography column. The fused silica capillary column used with Method 625.1 provides superior chromatographic resolution of components compared to a packed column, and this undoubtedly contributes to greater accuracy with complex environmental samples. The advantages of superior chromatographic resolution have been discussed by many authors (*1*).

Method Precision. The total method precision, as expressed in the estimated standard deviations of the percentage recoveries, is a function of the precision of many individuals method steps. Variabilities in the solvent extraction, extract concentration, and internal standard addition to the concentrated extract all contribute to the overall precision. One variable present in the GC/MS analysis using the repetitive measurement of spectra method of data acquisition is illustrated in Figure 5. The peak profile shown represents the real concentration of any component as it enters and leaves the ion source of the mass spectrometer. The series of diagonal line segments below the profile represents the repetitive sweep of the mass spectrometer through the 45–450 amu mass range. The points on the line segments represent the mass of the single ion used for the peak area measurement. The corresponding points on the real concentration profile represent the concentration at which the quantitation ion's abundance is measured. Since the mass spectrometer sweep is at regular intervals, but the concentration maximum may occur at any random time, there is no assurance that a quantitation ion's abundance will be measured when the maximum concentration of the substance is in the mass spectrometer. Therefore, peak areas, rather than peak heights, are used as a more consistent measure of compound concentration.

The precision of the area measurement depends on the number of points per peak, and the number of points depends on the spectrometer sweep time, the width of the peak, and the number of ions used to measure each compound. One quantitation mass was selected for each compound except toxaphene to minimize contributions from coeluting or background materials that generate ions with masses common to those in the spectrum of the analyte. With Method 625, the spectrometer sweep time of 4.3 s allowed at least six measurements of the abundance of the quantitation mass 99% of the time. Frequently seven or more points were obtained per peak since many peaks were 30 s wide at the base line. With Method 625.1, the spectrometer sweep time of 1 s allowed at least five measurements of the abundance of the quantitation ion.

It is very difficult to determine the extent that variations in area measurements affect total method precision, because area variabilities are not easily separated from variabilities in making standard solutions, syringe filling, injection, and chromatography. However, the sum of these variabilities can be compared to the total method precision, which includes the variations in extraction efficiency and extract processing. With packed columns and on-column injection, careful, experienced, and skilled operators achieve relative standard deviations (RSD) in the range of 2–7% for the measurement of response factors (*13*). With fused silica capillary columns, 1 s sweep times, and splitless injection RSDs in the range of 4–12% have been reported (*14*) for the measurement of response factors for most analytes. In the latter study the mean RSD was 11.4%, and this included a number of the same problem analytes such as *N*-nitrosodimethylamine, benzidine, etc. discussed in this paper

For Method 625 the mean RSD for concentration measurements computed from the data in Tables I–VII is 21%. For Method 625.1 this estimate of mean total method precision is 20%. In computing these means the measurement s of four compounds with standard deviations greater than 67% were excluded. It appears that, on average, at least half of the total method variability results from the sample extraction and processing steps, and for some compounds, sample preparation may account for as much as 75% of the total method variability.

CONCLUSION

The accuracy and precision data presented in this paper serve as base lines for comparison of alternative analytical methods and the estimation of matrix effects. The reagent water data obtained with a single calibration point at nearly the same concentration as the analytes probably represents the maximum performance of the methods. With real environmental samples a multipoint calibration is used, and no significant loss in accuracy or precision is expected as long as the samples have matrices similar to the reagent water, that is, surface water, groundwater, and drinking water. Significantly different method performance is expected with samples that are highly contaminated with water-soluble organic solvents or other materials that dramatically change the sample matrix. In one reported study (*11*) Method 625 was used to measure some of the same analytes in reagent water and in a diverse group of wastewaters. Although many compounds showed depressed mean recoveries in wastewater, a surprising number did not, including some substituted phenols. The pH conditions of Method 625.1 are expected to reduce the emulsion problems associated with Method 625 and wastewater (*11*), and this should reduce the observed matrix effects for some compounds. The application of the method of standard additions or adoption of an isotope dilution calibration procedure (*15*) are approaches to improved accuracy and precision which could be implemented with these methods to overcome sample matrix effects.

The application of the selected ion monitoring (SIM) method of data acquisition could provide improved peak area measurement accuracy and precision. Also improved sensitivity is available with SIM, but the loss of qualitative information is significant and usually unacceptable. With repetitive measurements of complete mass spectra, more than one ion could be used for quantitative analysis, but this is not expected to improve sensitivity by more than 50% since one of the two ions is usually the base peak. Finally, method detection limits may be estimated by using the data in Tables I–VII and a published procedure (*16*). These methods detection limits generally fall in the range of 1–10 μg/L.

Overall, Methods 625 and 625.1 allow well-supported identifications of large numbers of compounds in water samples. However, the precision and accuracy of the concentration measurements are generally less than those which may be achieved with the selected ion monitoring method of data acquisition and isotope dilution calibration (*1*).

ACKNOWLEDGMENT

The authors express their appreciation to William Middleton, Jr., who provided excellent technical assistance in preparing standard solutions and conducting most of the sample processing prior to analysis.

Registry No. Water, 7732-18-5; 1,2-dichlorobenzene, 95-50-1; 1,3-dichlorobenzene, 541-73-1; 1,4-dichlorobenzene, 106-46-7; 1,2,4-trichlorobenzene, 120-82-1; 1,2,3-trichlorobenzene, 87-61-6; 1,3,5-trichlorobenzene, 108-70-3; 1,2,3,4-tetrachlorobenzene, 634-66-2; 1,2,3,5-tetrachlorobenzene, 634-90-2; pentachlorobenzene, 608-93-5; hexachlorobenzene, 118-74-1; nitrobenzene, 98-95-3; 2,4-dinitrotoluene, 121-14-2; 2,6-dinitrotoluene, 606-20-2; phenol, 108-95-2; 2-fluorophenol, 367-12-4; 2-chlorophenol, 95-57-8; 2-nitrophenol, 88-75-5; 4-nitrophenol, 100-02-7; 2,4-dichlorophenol, 120-83-2; 2,4-dimethylphenol, 105-67-9; 2,4-dinitrophenol, 51-28-5; 4-chloro-3-methylphenol, 59-50-7; 2,3,6-trichlorophenol, 933-75-5; 2,4,6-trichlorophenol, 88-06-2; 3,4,5-trichlorophenol, 609-19-8; 2,4,6-trimethylphenol, 527-60-6; 2-methyl-4,6-dinitrophenol, 534-52-1; pentachlorophenol, 87-86-5; naphthalene, 91-20-3; acenaphthylene, 208-96-8; acenaphthene, 83-32-9; fluorene, 86-73-7; anthracene, 120-12-7; phenanthrene, 85-01-8; fluoranthene, 206-44-0; pyrene, 129-00-0; benz[*a*]anthracene, 56-55-3; chrysene, 218-01-9; benzo[*a*]pyrene, 50-32-8; benzo[*b*]fluoranthene, 205-99-2; benzo[*k*]fluoranthene, 207-08-9; benzo[*ghi*]perylene, 191-24-2; indeno[1,2,3-*cd*]pyrene, 193-39-5; dibenzo[*a,h*]anthracene, 53-70-3; hexachlorocyclohexane (α-isomer), 319-84-6; hexachlorocyclohexane (β-isomer), 319-85-7; hexachlorocyclohexane (γ-isomer), 58-89-9; hexachlorocyclohexane (δ-isomer), 319-86-8; aldrin, 309-00-2; endrin, 72-20-8; dieldrin, 60-57-1; heptachlor, 76-44-8; heptachlor epoxide, 1024-57-3; chlordane (γ-isomer), 5566-34-7; endosulfan I, 959-98-8; endosulfan II, 33213-65-9; endosulfan sulfate, 1031-07-8; toxaphene, 8001-35-2; dimethyl phthalate, 131-11-3; diethyl phthalate, 84-66-2; di(*n*-butyl) phthalate, 84-74-2; *n*-butyl benzyl phthalate, 85-68-7; di(*n*-octyl) phthalate, 117-84-0; di(2-ethylhexyl) phthalate, 117-81-7; *N*-nitrosodimethylamine, 62-75-9; *N*-nitrosodi(*n*-propyl)amine, 621-64-7; *N*-nitrosodiphenylamine, 86-30-6; isophorone, 78-59-1; hexachloroethane, 67-72-1; bis(2-chloroethyl) ether, 111-44-4; bis(2-chloroisopropyl) ether, 39638-32-9; bis(2-chloroethoxy)methane, 111-91-1; hexachlorobutadiene, 87-68-3; hexachlorocyclopentadiene, 77-47-4; 4-chlorophenyl phenyl ether, 7005-72-3; 4-bromophenyl phenyl ether, 101-55-3; 1-fluoronaphthalene, 321-38-0; 2-chloronaphthalene, 91-58-7; 4,4′-dibromobiphenyl, 92-86-4; 4,4′-dibromooctafluorobiphenyl, 10386-84-2; Arochlor 1221, 11104-28-2; Arochlor 1248, 12672-29-6; Arochlor 1254, 11097-69-1; benzidene, 92-87-5; 4,4′-diamino-3,3′-dichlorobiphenyl, 91-94-1; DDD, 72-54-8; DDT, 50-29-3; DDE, 72-55-9.

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Effect of C_{18} Surface Coverage on Selectivity in Reversed-Phase Liquid Chromatography of Polycyclic Aromatic Hydrocarbons

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Recent studies have reported selectivity differences for polycyclic aromatic hydrocarbons (PAH) on different octadecylsilane (C_{18}) reversed-phase liquid chromatographic columns. In this paper the differences in liquid chromatographic selectivity for various C_{18} columns were correlated with differences in C_{18} surface coverage data (as determined from elemental analysis and specific surface area measurements on the C_{18} modified silica). Significant differences in selectivity were observed for selected PAH and polyphenyl arenes on monomeric vs. polymeric C_{18} materials. Selectivity factors for 50 PAH, alkyl-substituted PAH, polyphenyl arenes, and polycyclic aromatic sulfur heterocycles were determined on columns from the same manufacturer from seven different lots of C_{18} material with different C_{18} surface concentrations. In addition to providing information concerning the contribution of C_{18} surface coverage to retention and selectivity in the LC separation of these compounds, these studies provide useful information regarding the selection of the appropriate column to optimize the separation of selected polycyclic aromatic compounds.

Reversed-phase liquid chromatography (LC) on chemically bonded octadecylsilane (C_{18}) stationary phases is by far the most popular LC mode for the separation of polycyclic aromatic hydrocarbons (PAH). Reversed-phase LC on C_{18} phases provides excellent selectivity for the separation of PAH isomers and alkyl-substituted PAH isomers (*1*). Recently, reversed-phase LC on C_{18} columns has been specified as the method of choice for the analysis of aqueous effluents for the determination of the 16 PAH on the U.S. Environmental Protection Agency's priority pollutant list (*2*). In addition to the environmental interest in the determination of PAH by LC, PAH are excellent nonpolar solutes for use in the investigation of reversed-phase LC retention mechanisms.

Since Schmit et al. (*3*) first described the separation of PAH on a chemically bonded C_{18} phase in 1971, numerous reports of LC retention data for PAH on various C_{18} columns have appeared. Recent studies by Wise et al. (*4*), Ogan and Katz (*5*, *6*), Colmsjö and MacDonald (*7*), and Amos (*8*) found that C_{18} columns from various manufacturers not only provided different separation efficiencies but often provided different selectivities and retention characteristics for PAH. In these studies, attention was focused on three groups of PAH isomers which are difficult to separate: benz[*a*]anthracene and chrysene; benzo[*e*]pyrene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, and benzo[*k*]fluoranthene; and benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene. All of these studies found that different C_{18} columns provided somewhat different selectivities for PAH and that under the experimental conditions of these studies (i.e., acetonitrile and water mixtures as the mobile phase), only one type of polymeric C_{18} material studied was successful in completely resolving all of these difficult to separate PAH isomers. In comparing efficiencies, Amos (*8*) found that the polymeric material which provided the greatest resolution was one of the lowest in column efficiency. Goldberg (*9*) compared the capacity factor (k') for anthracene and the selectivity factor (α) for anthracene/naphthalene on 31 different commercial reversed-phase columns. Selectivity differences for columns from the same manufacturer but from different production lots have also been observed (*5*, *6*, *10*). Selectivity differences on various C_{18} columns for other solutes (e.g., steroids and PAH metabolites) have also been reported (*11–13*).

The differences in chromatographic retention and selectivity as described in these studies (*4–13*) are a result of the utilization of different silica materials as supports and a variety of reagents and procedures to produce the C_{18} bonded phase. Several recent reports have reviewed the state of the art of chemically bonded alkyl reversed-phase packing materials (*14–17*). A number of workers have examined the parameters which may affect the chromatographic characteristics of alkyl bonded materials, i.e., carbon content, length of alkyl chain, surface area, and number of unreacted silanol groups (*16*, *18–28*). Several studies have shown that the capacity ratio, k', generally increases with increasing carbon content (*17*, *28*). However, as pointed out by Unger et al. (*29*), carbon content alone is often misleading because of differences in the surface area of the original silica which results in different surface coverage (or concentration) of the bonded alkyl groups (μmol/m^2).

Most of the data in the literature for surface concentration (density) of bonded alkyl groups [Unger et al. (*27*, *29*), Berendsen et al. (*20*, *21*), Karch et al. (*18*), and Hemetsberger et al. (*22*, *24*)] have been calculated by using specific surface area of the underivatized silica with a correction factor for the contribution of the organic layer to the weight of the chemically modified silica as shown in the following equation:

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